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Predictors of survival in restrictive chronic lung allograft dysfunction after lung transplantation

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KEYWORDS:
chronic lung allograft dysfunction;
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BACKGROUND: Chronic lung allograft dysfunction (CLAD) is the main factor limiting long-term survival after lung transplantation. Besides bronchiolitis obliterans syndrome, a restrictive phenotype of CLAD (rCLAD) exists, which is associated with poor prognosis after diagnosis. However, survival determinants for rCLAD remain to be elucidated. Our aim in this study was to establish parameters predicting survival in patients with rCLAD.

METHODS: All patients diagnosed with rCLAD in 2 lung transplant centers were assessed in a retrospective manner. Various clinical parameters [demography, pulmonary function, bronchoalveolar lavage (BAL), histopathology, radiology and blood differentials] at rCLAD diagnosis were correlated with graft survival using unadjusted and adjusted analysis.

RESULTS: A total of 53 patients with rCLAD were included with a median graft survival after diagnosis of 1.1 years. Univariate analysis demonstrated that lower-lobe-dominant or diffuse infiltrates on chest computed tomography, presence of an identifiable trigger before rCLAD onset, lymphocytic bronchiolitis, increased BAL neutrophilia, increased BAL eosinophilia and increased blood eosinophils were associated with inferior graft survival after rCLAD diagnosis. Multivariate analysis confirmed the association of location of infiltrates and blood eosinophilia on graft survival.

CONCLUSION: In this study we have identified parameters associated with graft survival after rCLAD diagnosis that may be useful to predict prognosis.

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Chronic lung allograft dysfunction (CLAD) remains the main factor limiting long-term survival after lung transplantation and contributes to inferior survival when compared with other types of solid-organ transplantation.[1] CLAD is used to describe patients with an irreversible decline in forced expiratory volume in 1 second (FEV_1), which, upon ruling out other causes, may be due to chronic graft rejection.[2] Approximately 30% of patients who develop CLAD in the framework of chronic rejection will develop a restrictive pulmonary function defect (rCLAD).[3] Typical radiologic and histopathologic characteristics of rCLAD include persistent

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infiltrates as well as (sub)pleural and septal thickening on chest computed tomography (CT), and pleuroparenchymal fibroelastosis and concurrent obliterative bronchiolitis (OB) on histopathologic examination.[4–6] In contrast, patients with so-called bronchiolitis obliterans syndrome (BOS) demonstrate a strictly obstructive pulmonary function decline with typical air trapping and mosaic attenuation on chest CT, whereas histopathology demonstrates OB but otherwise normal lung parenchyma. Importantly, rCLAD patients have a significantly worse prognosis (6 to 18 months) after diagnosis when compared to patients with BOS (median survival 3 years).[4–6] The determinants of this inferior survival rate with rCLAD are currently unknown. On the contrary, Finlen-Copeland et al showed that there are specific determinants of survival for patients with BOS, and we aimed to determine whether similar predictors of poor survival are present in rCLAD.[7]

The aim of this study is to determine the association between routinely measured parameters on demography, pulmonary function, lavage, histopathology, radiology and blood differentials at the moment of diagnosis as well as subsequent survival after rCLAD diagnosis.

Methods

Patient selection

This retrospective study, performed at the University Hospital UZ Gasthuisberg, Leuven, Belgium, and the University Hospital of Zurich, Switzerland, included all patients with single, double or heart-lung transplantation with a diagnosis of rCLAD. All patients had sufficiently long survival to have a certain CLAD phenotype and an impact on the analysis (i.e., 1 year). The study period ended July 15, 2015. The local hospitals' ethics committees approved the study (s51577, s54738 and 2013-0285). In both cohorts, patients with CLAD (defined as a persistent decrease in FEV_1 of $\geq$20% in the absence of other causes) were further sub-divided into BOS or rCLAD, as previously described: decrease in total lung capacity (TLC) $\geq$10% compared with baseline with concomitant persistent infiltrates on high-resolution CT (HRCT) or when TLC was not available; a decrease in FVC of $\geq$20% compared with the best post-operative values was used in combination with presence of concomitant persistent infiltrates on HRCT to diagnose rCLAD.[6] Patients not fulfilling these criteria were diagnosed with BOS and then excluded from further analysis. For patients who evolved from BOS to rCLAD (as previously described by Sato et al[5]), the time of diagnosis of rCLAD was used for further analysis, whereas previous BOS was assessed as a separate (binary) parameter. Because we were also interested in survival after rCLAD diagnosis in single lung transplant recipients, diagnosis was made using an increase in FEV_1/FVC of $\geq$20%, with concomitant persistent infiltrates in the transplanted lung.[6] HLA antibody detection was performed using the microbead array solid-phase (Luminex) method.

Study parameters

CT was performed at rCLAD diagnosis and the dominant distribution of infiltrates at the moment of rCLAD diagnosis was scored by a single observer as either apical, basal or diffuse. Patient variables were collected and their effect on survival was assessed. Re-transplantation was considered as graft loss/death. Our primary aim was to assess the effect of study parameters on graft survival (death or re-do lung transplantation).

Bronchoalveolar lavage (BAL) with accompanying transbronchial biopsies (TBBs) was performed within 2 months of diagnosis of rCLAD using 2 × 50 ml of saline, as described elsewhere.[8] The returned BAL fractions were pooled and processed for total and differential cell counting and routine clinical microbial screening. TBBs were assessed for acute rejection (Grade A) or lymphocytic bronchiolitis (Grade B), according to guidelines of the International Society for Heart and Lung Transplantation (ISHLT).[9]

Statistics

Results are presented as median (interquartile range). Survival was compared using Kaplan-Meier curves and log-rank analyses. Group differences were compared using Fisher's exact test, whereas continuous variables were compared using the Mann-Whitney *U*-test. Statistical analyses were performed with GraphPad Prism, version 6.0 (GraphPad, Inc., La Jolla, CA). Unadjusted analysis with continuous variables and adjusted analysis using a Cox model (PHREG procedures) were performed with SAS, version 9.3. $p < 0.05$ was considered significant.

Results

Patients' characteristics

In Leuven, CLAD was diagnosed in 159 of 593 patients (26.8%), rCLAD was diagnosed in 40 patients (25%), of whom 2 were excluded for further analysis because follow-up was not long enough to provide certainty of phenotype and impact on analysis (i.e., <1 year), resulting in a total study population of 38 patients. In the Zurich cohort, 126 of 351 patients (36%) were diagnosed with CLAD, of whom 15 were diagnosed with rCLAD (12%). There was no difference in graft survival after rCLAD diagnosis between the 2 cohorts ($p = 0.25$). To increase statistical power, we opted to combine both patient cohorts for further analysis. Detailed patient characteristics are summarized in Table 1, separately for the 2 cohorts. Consequently, a total of 53 patients were included in this study. Of these 53 patients, 28 were diagnosed using TLC and 25 were diagnosed using FVC decline and FEV_1/FVC ratio and the presence of concomitant radiologic infiltrates. Interestingly, in 15 of 47 patients assessed (32%), donor-specific anti-HLA antibodies were positive at diagnosis of rCLAD. These antibodies were exclusively Type II and, although we did not have more specific data for 1 patient, these were mostly directed against DQ (12 of 14), with 4 of 14 being DR-positive (2 patients were both DQ-positive and DR-positive). In total, 10 were new-onset cases, whereas 5 were positive before rCLAD diagnosis.

Eleven patients (21%) were alive at the end of the study, whereas 30 (57%) died and 12 (23%) underwent lung re-transplantation. Median graft survival after diagnosis was 1.1 years. One-, 3- and 5-year graft survival was 55%, 24% and 6%, respectively (Figure 1). Only 8 patients were not treated with macrolides at the time of rCLAD, of whom 4 had never undergone macrolide therapy and 4 were initiated >1 month after diagnosis. Fourteen patients were initiated at the moment of diagnosis, whereas the remaining patients ($n = 31$)

Table 1 Patients' Characteristics of 53 rCLAD Patients Stratified According to LTx Cohort

Characteristics	Leuven	Zurich
Number of patients	38	15
Age at diagnosis (years)	55 (31-62)	61 (57-64)
Native lung disease (*n*)	15 COPD, 10 ILD, 6 CF, 6 PHT, 1 other	3 COPD, 8 ILD, 3 CF, 1 PHT
Gender male [*n* (%)]	19 (50%)	12 (80%)
Type of transplant (*n*)	30 SSLTx, 4 HLTx, 4 SLTx	13 SSLTx, 2 SLTx
Time between LTx and diagnosis (days)	1,196 (589-1,685)	467 (266-898)
Outcome		
Redo lung transplant [*n* (%)]	12 (32%)	0 (0%)
Death [*n* (%)]	16 (42%)	14 (93%)
Alive [*n* (%)]	10 (26%)	1 (7%)
Lung function		
Previous BOS [*n* (%)]	16 (41%)	12 (80%)
FEV_1 at diagnosis (liters)	1.46 (1.12-2.23)	1.85 (1.11-2.09)
FEV_1 (% of best)	58 (46-68)	59 (54-71)
66% to 80% (*n*)	13 (34%)	5 (33%)
50% to 65% (*n*)	14 (37%)	7 (47%)
$<50\%$ (*n*)	11 (29%)	3 (20%)
FVC at diagnosis (liters)	2.26 (1.59-2.89)	2.17 (1.44-3.08)
FVC (% of best)	66 (53-74)	63 (56-71)
TLC at diagnosis (liters)	4.01 (3.11-5.65)	NA
TLC (% of best)	78 (75-84)	NA
Identifiable trigger [*n* (%)]	17 (45%)	7 (47%)
Anti-HLA antibodies positive [*n* (%)]	12 of 32 (38%)	4 (27%)
Transbronchial biopsy		NA
Acute rejection [*n* (%)]	8 of 27 (30%)	
Lymphocytic bronchiolitis [*n* (%)]	8 of 32 (25%)	
BAL at diagnosis (33 of 38)		NA
Total cell count	0.12 (0.06–0.32)	
Macrophages (%)	73 (47–84)	
Lymphocytes (%)	9 (3–19)	
Neutrophils (%)	12 (6–25)	
Eosinophils (%)	0.6 (0.0–3.6)	

Data presented as number (%) or median (25-75% IQR).

BAL, bronchoalveolar lavage; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; PHT, pulmonary hypertension; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HLA, human leukocyte antigen; HLTx, heart–lung transplantation; ILD, interstitial lung disease; LTx, lung transplantation; NA, not available; SLTx, single lung transplantation; SSLTx, sequential single lung transplantation.

were already on long-term macrolide therapy for a longer period (>1 month). Patients already on long-term macrolide therapy tended to have a better survival after rCLAD diagnosis compared with the non-treated patients ($p = 0.076$). In case of rCLAD progression, given the lack of effective therapy, some patients received "compassionate-use" therapy (pirfenidone, $n = 6$; plasmapheresis, $n = 5$; rituximab, $n = 3$, extracorporeal photopheresis, $n = 1$). Plasmapheresis or pirfenidone therapy was not associated with better survival ($p = 0.73$ and $p = 0.57$, respectively).

Demographic factors and survival

Age ($p = 0.79$), gender ($p = 0.29$), native lung disease ($p = 0.62$), type of transplant (single vs sequential single vs heart-lung, $p = 0.96$) and time after lung transplant to rCLAD diagnosis ($p = 0.55$) did not influence survival after diagnosis. More details regarding univariate analysis are shown in Table 2. Patients who developed rCLAD within the first 2 years after transplantation ($n = 23$, 43%) had similar survival compared with patients developing rCLAD >2 years after transplantation ($n = 30$, $p = 0.72$). Twenty-four patients (45%) had a distinguishable trigger shortly before rCLAD onset. These triggers were present within 2 weeks before diagnosis and required admission to the ward or an additional outpatient clinic visit. Infection was present in 14 patients (cytomegalovirus, $n = 6$; para-influenza virus, $n = 2$; fusarium, $n = 1$, no pathogen detected but initial response to antibiotics, $n = 5$), acute respiratory distress syndrome (ARDS) in 3 patients, high-grade acute cellular rejection in 2 patients and acute antibody-mediated rejection in 5 patients. All other patients evolved to rCLAD without an identifiable cause ($n = 29$). Patients with a discernible cause/trigger of rCLAD have worse outcome compared with patients without specific triggers [$p = 0.0063$, hazard ratio (HR) 2.410, 95% confidence interval (CI) 1.283 to 4.529]. The presence of donor-specific anti-HLA antibodies did not correlate with survival after diagnosis ($p = 0.30$).

Pulmonary function parameters and survival

Patients initially diagnosed with BOS but with a later progression to rCLAD ($n = 28$, 53%) had similar graft

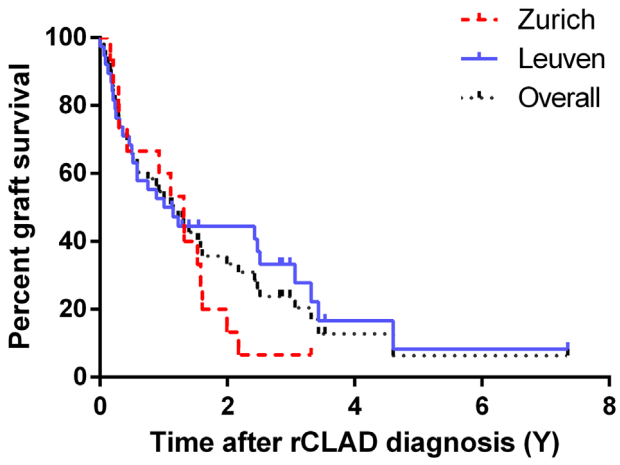

Figure 1 Graft loss in patients diagnosed with rCLAD for the Zurich, Leuven and total cohorts. Median time to graft loss was 1.1 years in the total cohort. One-, 3- and 5-year survival was 55%, 24% and 6%, respectively.

survival compared with patients developing rCLAD immediately ($p = 0.23$). Similarly, FEV_1/best FEV_1 at diagnosis of rCLAD (FEV_1/best FEV_1 66% to 80% vs 50% to 65% vs <50%) did not influence survival ($p = 0.38$). When comparing CLAD patients with high-grade onset (FEV_1 at diagnosis <65% of best FEV_1, $n = 33$) and those with low-grade onset (FEV_1 at diagnosis 65% to 80% of best FEV_1, $n = 20$), there was no significant survival difference ($p = 0.52$). When using percent best FEV_1 and FVC at the moment of rCLAD diagnosis, we did not observe an association with survival ($p = 0.91$ and $p = 0.62$, respectively). In addition, FEV_1/FVC, best FEV_1 and FVC did not impact survival ($p = 0.15$, $p = 0.34$ and $p = 0.22$, respectively); TLC at the moment of diagnosis was available in 28 patients only and was also not associated with survival ($p = 0.24$).

BAL and biopsy analysis and survival

BAL at the time of rCLAD diagnosis was available in 33 of 53 patients. Total BAL cell count ($p = 0.29$) and percent BAL lymphocytes ($p = 0.54$) did not influence graft survival after diagnosis. The percent neutrophils ($p = 0.017$, HR 1.018, 95% CI 1.003 to 1.032 for every percent increase) and percent eosinophils ($p = 0.033$, HR 1.029, 95% CI 1.002 to 1.055 for every percent increase) were associated with inferior survival after diagnosis, whereas the percent macrophages ($p = 0.0008$, HR 0.975, 95% CI 0.961 to 0.990 for every percent increase) was associated with an improved survival. Analysis of BAL eosinophilia as a binary variable (≥2%, $n = 12$ vs <2%, $n = 21$) revealed that there was a strong association with increased eosinophilia and survival after diagnosis ($p = 0.0002$, HR 6.179, 95% CI 2.345 to 16.282). Similarly, analysis with a binary variable for BAL neutrophilia (>10%, 17 patients vs <10%, 16 patients) showed that BAL neutrophilia >10% was associated with worse outcome ($p = 0.019$, HR 2.723; 95% CI 1.182 to 6.271). Because follow-up BAL was only available in a small subset of patients ($n = 10$), no further follow-up analysis was performed.

Table 2 Summary of Effects of Different Covariates on Survival After Diagnosis of rCLAD in the Combined Cohort ($n = 53$)

Covariates	HR	95% CI	*p*-value
Female gender	0.714	0.380 to 1.339	0.29
Age at onset	0.997	0.977 to 1.017	0.79
Native disease			0.62
COPD		Reference	
ILD	1.548	0.735 to 3.263	0.25
PHT	1.337	0.474 to 3.770	0.58
Cystic fibrosis	1.686	0.685 to 4.151	0.26
Type of transplant			0.96
SLTx	1.013	0.355 to 2.885	0.98
SSLTx			
HLTx	1.199	0.362 to 3.974	0.77
Early onset	1.153	0.529 to 2.512	0.72
Trigger before rCLAD	2.410	1.283 to 4.592	**0.0063**
Previous BOS	0.685	0.368 to 1.274	0.23
Anti-HLA antibodies	1.461	0.708 to 3.012	0.30
FEV_1/FEV_1 best			0.38
66% to 80%	1.063	0.480 to 2.354	0.88
50% to 65%	1.637	0.729 to 3.679	0.23
<50%		Reference	
Pulmonary function			
FVC/FVC best	0.606	0.086 to 4.284	0.62
FEV_1/FVC	4.923	0.551 to 4.018	0.15
BAL			
Total cell count	1.836	0.589 to 5.724	0.29
Macrophages (%)	0.975	0.961 to 0.990	**0.0008**
Neutrophils (%)	1.018	1.003 to 1.032	**0.0169**
Lymphocytes (%)	1.013	0.973 to 1.054	0.54
Eosinophils (%)	1.029	1.002 to 1.055	**0.033**
Biopsy			
No acute rejection	1.779	0.804 to 3.937	0.27
LB	2.427	1.015 to 5.814	**0.046**
Imaging (infiltrates)			**0.0021**
Upper		Reference	
Lower	2.367	1.153 to 4.836	**0.0181**
Diffuse	4.077	1.682 to 9.883	**0.0019**

Data were complete for all variables except BAL ($n = 33$), acute rejection ($n = 27$) and LB ($n = 32$). Bold *p*-values are statistically significant ($p < 0.05$). BAL, bronchoalveolar lavage; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HLA, human leukocyte antigen; HLTx, heart–lung transplantation; ILD, interstitial lung disease; LB, lymphocytic bronchiolitis; PHT, pulmonary hypertension; Reference, reference group, against which the HRs of the other groups are shown; SSLTx, sequential single lung transplantation; SLTx, single lung transplantation.

Interpretable biopsy results for Grade A rejection at the time of rCLAD diagnosis were available for 27 patients, of which 8 (29%) indicated acute rejection (4 with Grade A1, 1 with A2, 2 with A3, 1 with A4). Thirty-two of 38 biopsies could be graded for lymphocytic bronchiolitis (LB), of which 8 (26%) were positive (8 with Grade B1R). Grade A rejection at diagnosis did not influence survival ($p = 0.093$), although LB did [patients with LB had worse survival ($p = 0.046$, HR 2.428, 95% CI 1.015 to 5.804)]. Because all these patients were treated with high-dose intravenous

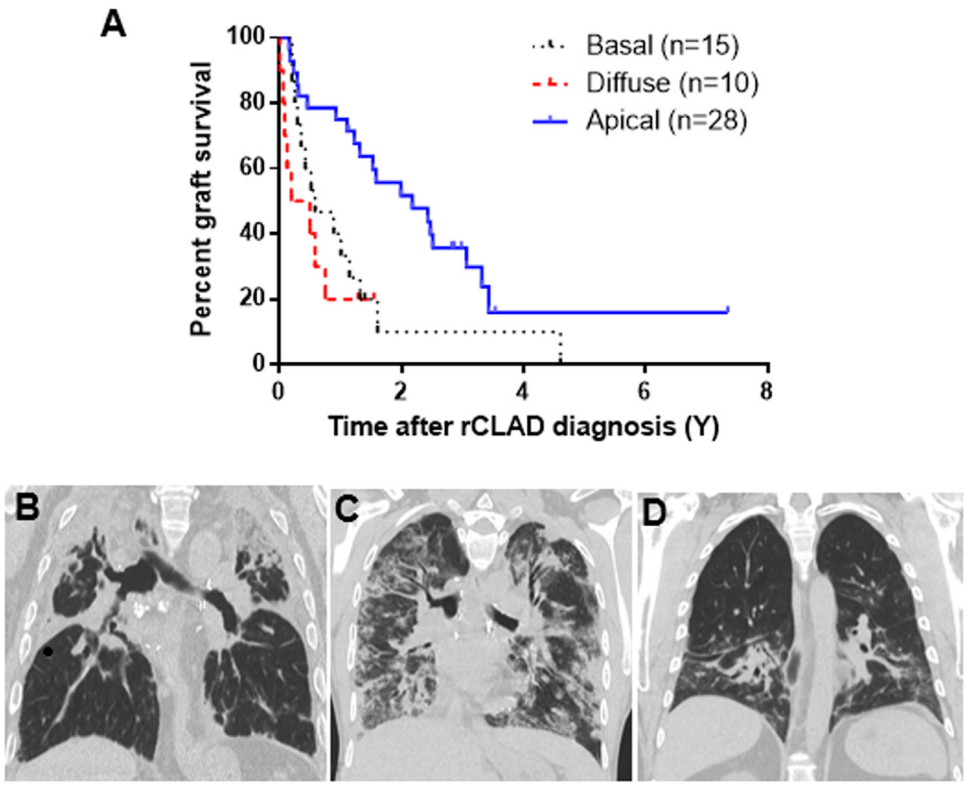

Figure 2 (A) Graft loss in patients diagnosed with rCLAD sub-divided based on the localization of CT infiltrates at the moment of rCLAD diagnosis. Better survival was observed in patients with upper lobe infiltrates compared with diffuse or lower lobe infiltrates ($p = 0.0021$). Median survival was 2.2 years, 0.6 year and 0.3 year for apical, basal and diffuse presence of CT infiltrates, respectively. (B) Representative example of high-resolution CT (HRCT) chest scan with upper lobe predominant infiltrates. (C) Example of HRCT scan with diffuse infiltrates. (D) Representative example of HRCT scan with lower lobe predominance.

steroids and had no improvement in pulmonary function, post-hoc diagnosis of rCLAD was made regardless of the initial finding of Grade A rejection or LB on biopsy. Consequently, no follow-up biopsies were taken.

Imaging and survival

Discrimination was made based on localization of the infiltrates on CT scan at the moment of rCLAD diagnosis: upper-lobe–dominant vs lower-lobe-dominant and diffuse.[10] Twenty-nine of 53 patients (55%) demonstrated upper-lobe-dominant infiltrates, 14 of 53 (26%) lower-lobe-dominant infiltrates and 10 (19%) diffuse infiltrates. Interestingly, patients with upper-lobe–dominant infiltrates had significantly better graft survival compared with lower-lobe–dominant and diffuse infiltrates ($p = 0.0021$; Figure 2). Median graft survival in patients with upper-lobe-dominant infiltrates was 2.2 years as compared with 0.6 year in lower-lobe–dominant infiltrates and 0.3 year in diffuse infiltrates. By contrasting upper-lobe-dominant infiltrates vs lower-lobe and diffuse infiltrates together, the observation that patients with upper-lobe-dominant infiltrates had better survival was strengthened ($p = 0.0015$, HR 3.032; 95% CI 1.529 to 6.012).

Blood eosinophilia and survival

We also assessed the association between blood eosinophils and survival due to the important association between BAL eosinophils and graft survival after rCLAD diagnosis. Percent BAL eosinophilia correlated with total blood eosinophil count ($p = 0.0017$ and $R = 0.52$) and percent eosinophils ($p = 0.0004$ and $R = 0.58$). Mean blood eosinophil count at time of rCLAD diagnosis was $330 \pm 64 \times 10^6$/liter. Elevated blood eosinophil count was associated with worse survival after rCLAD diagnosis ($p = 0.0072$, HR 1.894, 95% CI 1.188 to 3.018). Receiver operator characteristic (ROC) analysis showed that a threshold of blood eosinophilia of $>240 \times 10^6$/liter had 71% sensitivity and 86% specificity to predict 1-year mortality (Figure 3A). Consequently, sub-division of the patient cohort based on total blood eosinophil count $>240 \times 10^6$/liter ($n = 21$) and $\leq 240 \times 10^6$/liter ($n = 32$) showed significantly worse survival in patients with elevated blood eosinophil counts ($p = 0.0015$, HR 3.03, 95% CI 1.57 to 5.84) (Figure 3B).

Ten patients showed a combination of basal or diffuse infiltrates and elevated blood eosinophil counts on CT. These patients showed significantly worse survival compared with the other patients ($n = 43$) ($p < 0.0001$, HR 8.85, 95% CI 3.57 to 22.01).

Adjusted analysis

We performed adjusted analysis using all significant variables that were available from the entire cohort from univariate analysis (trigger vs no trigger, upper-lobe-dominant

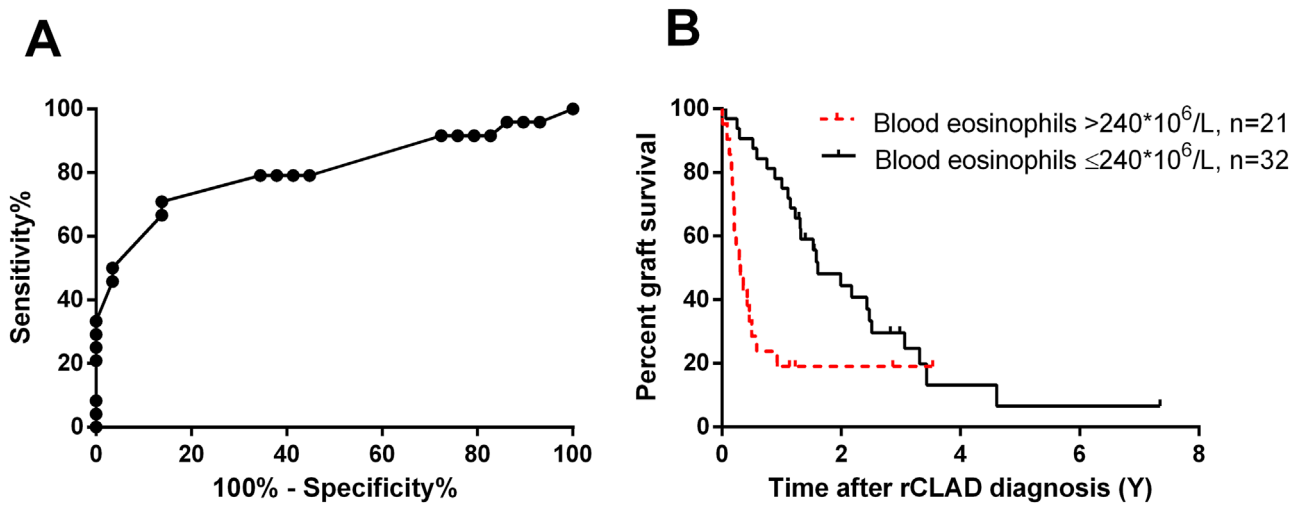

Figure 3 (A) ROC curve for 1-year mortality after rCLAD diagnosis based on absolute blood eosinophil count. Absolute blood eosinophilia was a good predictor of 1-year mortality ($p = 0.0001$, area under the curve = 0.80). Blood eosinophilia $>240 \times 10^6$/liter had 71% sensitivity and 86% specificity to predict 1-year survival. (B) Log-rank comparison of patients with absolute eosinophil count $>240 \times 10^6$/liter and $\leq 240 \times 10^6$/liter.

infiltrates vs lower-lobe-dominant/diffuse infiltrates, and absolute blood eosinophil count $>240 \times 10^6$/liter vs $\leq 240 \times 10^6$/liter). Discernible trigger proved to be no longer significant in this model ($p = 0.19$), whereas basal/diffuse infiltrates and absolute blood eosinophil count $>240 \times 10^6$/liter were associated with worse outcome ($p = 0.0026$ and $p = 0.0012$, respectively). To rule out the possibility that the observed effects were driven solely by a single center, we also added a binary center variable to the model. Center did not influence the observed associations between apical vs basal and diffuse infiltrates and absolute eosinophil counts ($p = 0.0015$ and $p = 0.0005$), whereas trigger was also not associated with graft survival ($p = 0.44$).

Discussion

Restrictive CLAD has recently been acknowledged as a novel and devastating phenotype of CLAD. The poor prognosis of rCLAD has been confirmed in several independent cohorts using different diagnostic criteria.[4–6] Therefore, we aimed to determine whether there are significant predictors of graft survival in rCLAD patients. Increased BAL neutrophilia or BAL eosinophilia, serum eosinophilia, distribution of the CT infiltrates and the presence of a discernible trigger of rCLAD were associated with worse survival after diagnosis in univariate analysis. In the multivariate analysis, localization of infiltrates and blood eosinophil count showed a strong correlation with outcome after rCLAD diagnosis.

We found that patients with upper-lobe–dominant infiltrates have a median survival of 2.2 years, which is superior to the average prognosis indicated in previous studies (0.7 to 1.5 years).[3] Patients with lower lobe and diffuse infiltrates have worse prognosis (median survival of 0.6 year and 0.3 year). We speculate that there is a large overlap between patients with diffuse parenchymal infiltrates and the recently described acute fibrinoid organizing pneumonia (AFOP)[11] as diagnosed by pathologic examination of biopsy specimens, whereas CT typically demonstrates bilateral infiltrates and pulmonary function shows restriction. Time to death of AFOP patients was reported to be 0.3 year in the study by Paraskeva et al, whereas, in our study, survival of rCLAD patients with diffuse infiltrates was also 0.3 year. Interestingly, from 3 patients with diffuse CT infiltrates in our cohort, the pathologic specimen at re-do transplantation confirmed AFOP diagnosis. Given these clinical similarities in survival and prognosis, we speculate that AFOP may reflect a subtype of rCLAD with the worst prognosis.

To our knowledge, this is the first time that survival determinants in patients with rCLAD were comprehensively assessed, although Sato et al already noted that progression of restrictive allograft syndrome (RAS) in patients typically shows a stepwise pattern.[12] Surprisingly, none of the pulmonary function variables investigated showed an association with survival after rCLAD diagnosis. Whereas longitudinal monitoring of pulmonary function has shown that decline in FVC is associated with poor outcomes in lung transplant recipients and in patients with idiopathic pulmonary fibrosis (IPF), interestingly, FVC at the time of rCLAD diagnosis was not a predictor of survival in our cohort[13,14]; this was probably due to the concomitant airway obstruction in rCLAD patients that is not present in IPF.[15,16] Similarly, although severity (BOS Stage 2 or 3) and timing (<2 years) of BOS at initial presentation correlates with poor survival, this was not seen in our patients with rCLAD, suggesting that rCLAD is a distinct phenotype in which inflammation may play a major role.[17] The strong predictive effect of eosinophilia also confirms own previous research demonstrating that BAL eosinophilia $\geq 2\%$ predisposes to later development of CLAD, but more specifically rCLAD. We can now extend this further, however, and show that the BAL and blood eosinophils are associated with survival after rCLAD diagnosis as well.[8] Other potential survival determinants previously linked to BOS were also investigated in this population of rCLAD patients, but these were not found to be associated with poor prognosis.[18]

We observed that 45% of patients had an identifiable trigger preceding rCLAD. This proportion seems relatively low compared with the proportions described by the Toronto group, as they described an acute exacerbation (possible trigger) during follow-up of every RAS case analyzed.[12] On the other hand, this finding seems to be in line with the 33% described previously in BOS.[19]

Our study has some shortcomings, such as the relatively small number of patients, the heterogeneity of diagnostic criteria, and the large proportion of patients undergoing lung re-transplantation. However, rCLAD remains a relatively rare and under-studied disease. Nevertheless, the present study is the first to provide evidence of survival differences between rCLAD patients. The viability of our cohort is reflected in its similarity to the Toronto cohort, as the incidence of rCLAD (25% vs 30%), median survival (1.1 vs 1.5 years) and percentage of patients with upper lobe fibrosis were comparable (55% vs 41%). One could question the robustness of the used definition; however, the main parameters predicting survival seem to be both numeric (BAL and blood cellularity) and easy to interpret (acute trigger or location of radiologic infiltrates), making them useful for physicians, but still in need of validation by other centers. TLC was not consequently measured in all patients in the Leuven and Zurich cohort; therefore, both TLC and FVC criteria are used, which may introduce some bias, although comparing different diagnostic criteria was not an aim for our study. Last, we combined 2 different cohorts of lung transplant patients to increase statistical power, although there is no unified management approach between the 2 cohorts.

In conclusion, the present study may be a first indication of the different phenotypes of rCLAD with potentially major clinical implications. Patients with upper-lobe-dominant infiltrates have a survival rate similar to that of BOS patients. Consequently, a restrictive pulmonary function defect per se does not seem to be associated with worse prognosis, yet further investigation and validation is warranted.

Disclosure statement

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